PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P100653	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/SG2004/000352	International filing date *(day/month/year)* 25 October 2004	Priority date *(day/month/year)* 29 October 2003

International Patent Classification (IPC) or national classification and IPC

Int. Cl. ***C12Q 1/00*** (2006.01) ***C12Q 1/26*** (2006.01) ***G01N 27/26*** (2006.01)
C12Q 1/18 (2006.01) ***C12Q 1/54*** (2006.01) ***G01N 33/543*** (2006.01)

Applicant

AGENCY FOR SCIENCE, TECHNOLOGY AND RESEARCH et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 4 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of 7 sheets, as follows:
      - [ ] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - [X] Box No. I Basis of the report
   - [ ] Box No. II Priority
   - [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - [X] Box No. IV Lack of unity of invention
   - [X] Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - [ ] Box No. VI Certain documents cited
   - [ ] Box No. VII Certain defects in the international application
   - [ ] Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26 August 2005	12 January 2006
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **ALBERT S. J. YONG** Telephone No. (02) 62832160

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SG2004/000352

Box No. I Basis of the report

1. With regard to the language, this report is based on:

[X] The international application in the language in which it was filed

[ ] A translation of the international application into , which is the language of a translation furnished for the purposes of:

- [ ] international search (under Rules 12.3(a) and 23.1 (b))
- [ ] publication of the international application (under Rule 12.4(a))
- [ ] international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

[ ] the international application as originally filed/furnished

[X] the description:

pages **1-46,54(abstract)** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

[X] the claims:

pages as originally filed/furnished
pages* as amended (together with any statement) under Article 19
pages* **47-53** received by this Authority on **21 December 2005** with the letter of 21 December 2005
pages* received by this Authority on with the letter of

[X] the drawings:

pages **1/14 – 14/14** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

[ ] a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. [ ] The amendments have resulted in the cancellation of:

- [ ] the description, pages
- [ ] the claims, Nos.
- [ ] the drawings, sheets/figs
- [ ] the sequence listing *(specify)*:
- [ ] any table(s) related to the sequence listing *(specify)*:

4. [ ] This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- [ ] the description, pages
- [ ] the claims, Nos.
- [ ] the drawings, sheets/figs
- [ ] the sequence listing *(specify)*:
- [ ] any table(s) related to the sequence listing *(specify)*:

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SG2004/000352

Box No. IV Lack of unity of invention

1. ☐ In response to the invitation to restrict or pay additional fees the applicant has, within the applicable time limit:

   ☐ restricted the claims

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☐ neither restricted the claims nor paid additional fees

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is:

   ☐ complied with.

   ☒ not complied with for the following reasons:

   The International Preliminary Report on Patentability (Chapter II) has been drawn up in respect of the entire international application but the International Preliminary Examining Authority is of the opinion that the application does not appear to comply with the requirements of unity of invention as set forth in the PCT regulations (Article 34(3), Rule 68(1) PCT).

   The separate groups of invention are:

   1. Claims 1-24 and 43-44 relate to a sensor comprising a nanoparticulate membrane having dispersed therein an oxidoreductase and an electrochemical activator.

   2. Claims 25-42 relate to a redox polymer comprising a polymerisable ferrocene derivative and an acrylic acid derivative.

   Since the abovementioned groups of claims do not share any of the technical features identified, a "technical relationship" between the inventions, as defined in PCT rule 13.2 does not exist. Accordingly the international application does not relate to one invention or to a single inventive concept, a priori.

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos.

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-44	YES
	Claims		NO
Inventive step (IS)	Claims	1-44	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-44	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

CITATIONS

D1. US 5922183

D2. US 5922537

D3. BU, Anal. Chem. 68(22) 1996

D4. BU, Anal. Chem. 70(20) 1998

D5. US 4341881

NOVELTY AND INVENTIVE STEP

Claims 1-44: Claims 1-24 and 43-44 relate to a sensor comprising a nanoparticulate membrane having an oxidoreductase and an electrochemical activator dispersed therein, and claims 25-42 relate to a redox polymer comprising a polymerisable ferrocene derivative and an acrylic acid derivative.

None of the above citations disclose all of the features defined in the claims. D1 and D3 have been identified as the closest art. D1 differs from claims 1-24 and 43-44 in that the present sensor has an electrochemical activator diffusibly dispersed in a nanoparticulate membrane and that the sensor is stable even under prolonged periods of storage. D3 discloses a ferrocene containing redox gel, but it does not suggest the use of an acrylic acid derivative with low-pK_a end groups as defined in claim 25. Hence, the claims are novel and inventive.

What is claimed is:

1. A sensor for determining the presence of an analyte in a test sample, said sensor comprising:

a nanoparticulate membrane comprising nanoparticles of at least one inorganic oxide of an element selected from Group IA, IIA, IIIA, IVA, IB, IIB, IIIB, IVAB, VB, VIB, VIIB or VIIIB of the Periodic Table, and wherein an oxidoreductase and an electrochemical activator are diffusibly dispersed in said nanoparticulate membrane.

2. The sensor according to Claim 1, wherein the oxidoreductase is selected from the group consisting of glucose oxidase, hydrogen peroxidase, horseradish peroxidase, xanthine oxidase, cholesterol oxidase, hydrogen hydrogenase, lactate dehydrogenase, glucose dehydrogenase, NADH dehydrogenase, sarcosine oxidase, lactate oxidase, alcohol dehydrogenase, hydroxybutyrate dehydrogenase, glycerol dehydrogenase, sorbitol dehydrogenase, malate dehydrogenase, galactose dehydrogenase, malate oxidase, galactose oxidase, xanthine dehydrogenase, alcohol oxidase, choline oxidase, xanthine oxidase, choline dehydrohenase, pyruvate dehydrogenase, pyruvate oxidase, oxalate oxidase, bilirubin oxidase, glutamate dehydrogenase, glutamate oxidase, amine oxidase, NADPH oxidase, urate oxidase, cytochrome C oxidase, and actechol oxidase.

3. The sensor of Claim 1, wherein the electrochemical activator is a polymeric redox mediator capable of transferring electrons between the analyte and an electrode present in the sensor.

4. The sensor according to Claim 3, wherein the oxidoreductase is covalently linked to the polymeric redox mediator by cross-linkages.

5. The sensor according to Claim 1, wherein the element selected from Group IA, IIA, IIIA, IVA, IB, IIB, IIIB, IVAB, VB, VIB, VIIB or VIIIB of the Periodic Table is selected from the group consisting of aluminium, silicon, magnesium and zinc.

6. The sensor according to Claim 1, wherein the thickness of the membrane ranges from 250 to 500 µm.

7. The sensor according to Claim 6, wherein the size of the nanoparticles ranges from 10 nm to 1 µm.

8. The sensor according to Claim 1, wherein the membrane further comprises a polymeric binder.

9. The sensor according to Claim 8, wherein the polymeric binder is a polymer or copolymer comprising monomer units selected from the group consisting of vinyl pyridine, vinyl imidazole, acrylamide, acrylonitrile, and acrylhydrazide and acrylic acid.

10. The sensor according to Claim 1, further comprising:

a chamber for holding the test sample, said chamber being bounded at least between a working area on a working electrode and a working area on a reference electrode,

wherein the oxidoreductase and the electrochemical activator is coated on the working area of the working electrode.

11. The sensor according to Claim 10, wherein the working electrode comprises a material selected from the group consisting of gold, carbon, platinum, ruthenium dioxide, palladium, and conductive epoxies.

12. An electrically non-conductive, nanoparticulate membrane comprising nanoparticles of at least one inorganic oxide of an element selected from Group IA, IIA, IIIA, IVA, IB, IIB, IIIB, IVAB, VB, VIB, VIIB or VIIIB

of the Periodic Table, and wherein an oxidoreductase enzyme and an electrochemical activator are diffusibly dispersed in said nanoparticulate membrane.

13. The membrane of claim 12, wherein the electrochemical activator is a polymeric redox mediator capable of transferring electrons.

14. The membrane of claim 12, wherein the element selected from Group IA, IIA, IIIA, IVA, IB, IIB, IIIB, IVAB, VB, VIB, VIIB or VIIIB of the Periodic Table is selected from the group consisting of aluminium, silicon, magnesium and zinc.

15. The membrane of claim 12, wherein the thickness of the membrane ranges from 250 to 500 µm.

16. The membrane of claim 12, wherein the size of the nanoparticles ranges from 10 nm to 1 µm.

17. The membrane of claim 12, wherein the membrane further comprises a polymeric binder.

18. The membrane according to Claim 17, wherein the polymeric binder is a polymer or copolymer comprising monomer units selected from the group consisting of vinyl pyridine, vinyl imidazole, acrylamide, acrylonitrile, and acrylhydrazide and acrylic acid.

19. A process for producing a non-conductive, nanoparticulate membrane, said process comprising

mixing an electrochemical redox mediator with an oxidoreductase and nanoparticles of an inorganic oxide of an element from Group IA, IIA, IIIA, IVA, IB, IIB, IIIB, IVAB, VB, VIB, VIIB or VIIIB of the Periodic Table to form a nanocomposite ink; and

applying said nanocomposite ink onto a substrate.

20. The process of claim 19, wherein said nanocomposite ink is applied according to a predetermined pattern.

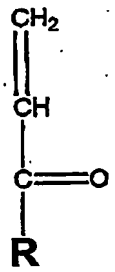
21. The process of claim 20, wherein said nanocomposite ink is applied by screen-printing.

22. The process according to claim 19, wherein the mixing further comprises mixing a polymeric binder into the nanocomposite ink.

23. The process according to claim 19, wherein the concentration of the electrochemical activator in the nanocomposite ink is about 15 mg/ml.

24. The process according to claim 19, wherein the concentration of enzyme in the nanocomposite ink is about 0.2 mg/ml.

25. A water soluble redox polymer comprising:
a first monomer unit comprising a polymerisable ferrocene derivative; and
a second monomer unit comprising an acrylic acid derivative having a primary acid functional group capable of acquiring a net charge, wherein the acrylic acid derivative is represented by the general formula (I)



wherein R is selected from the group consisting of $NH\text{-}C_nH_{2n}\text{-}PO_3H$ and $NH\text{-}C_nH_{2n}\text{-}SO_3H$, wherein the alkyl chain can be optionally substituted, and wherein n is an integer from 0 to 12.

26. The redox polymer of claim 25, wherein the polymerisable ferrocene derivative is selected from the group consisting of vinyl-ferrocene, acetylene-ferrocene, styrene-ferrocene and ethylene oxide-ferrocene.

27. The redox polymer of claim 26, wherein the ferrocene derivative is vinyl ferrocene.

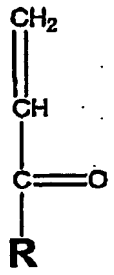
28. The redox polymer of Claim 25, wherein the molecular weight of the redox polymer is between about 1000 and 5000 Daltons.

29. The redox polymer of Claim 25, wherein the ferrocene loading in the redox polymer is between about 3% and 14%.

30. A process for preparing a water soluble, redox polymer, said process comprising:

polymerising a first monomer unit comprising a polymerisable ferrocene derivative with a second monomer unit comprising an acrylic acid derivative having an acid or base functional group capable of acquiring a net charge,

wherein the acrylic acid derivative is represented by the general formula (I)



wherein R is selected from the group consisting of $NH\text{-}C_nH_{2n}\text{-}PO_3H$ and $NH\text{-}C_nH_{2n}\text{-}SO_3H$, wherein the alkyl chain can be optionally substituted, and wherein n is an integer from 0 to 12,

wherein said polymerization is carried out in an aqueous alcoholic medium.

31. The process according to Claim 30, wherein the aqueous alcoholic medium comprises ethanol and water in a volumetric ratio of between about 2:1 and 3:1.

32. The process according to Claim 30, wherein the polymerization is initiated by adding a free radical initiator.

33. The process according to Claim 32, wherein the free radical initiator is selected form the group consisting of ammonium persulfate, potassium persulphate and sodium persulfate.

34. The process according to Claim 32 wherein the weight ratio of free radical initiator added is between about 20 mg to 40 mg per 1 gram of monomer.

35. The process according to Claim 30, wherein polymerization is carried out under reflux at a temperature of between about 60 °C to 80 °C.

36. The process according to Claim 31, wherein polymerization is carried out in an inert gas atmosphere.

37. The process according to Claim 30, wherein polymerization is carried out for about 24 hours.

38. The process according to Claim 30, further comprising forming a pre-reaction mixture prior to polymerizing said first and second monomers, comprising:
dissolving the acrylic acid derivative monomer unit in an aqueous alcoholic medium, then
adding the free radical initiator, and then
adding the polymerisable ferrocene derivative monomer unit to form the pre-reaction mixture.

39. The process according to Claim 38, wherein the feeding ratio of acrylic acid derivative to polymerisable ferrocene derivative in the pre-reaction mixture is between about 5 % and 15 % of the weight of monomer added.

40. The process according to Claim 38, wherein the polymerisable ferrocene derivative monomer unit is dissolved in an aqueous alcoholic medium prior to being added.

41. The process according to Claim 38, further comprising precipitating the redox mediator in an organic solvent.

42. The process according to Claim 38, wherein the organic solvent is selected from the group consisting of an ether and ketone.

43. The sensor according to claim 1, wherein the sensor is for determination of glucose concentration.

44. The use of a water soluble redox polymer as defined in any one of claims 25 - 29 as a redoxmediator for electrochemically determining the presence of an analyte in a test sample.